

Review

Molecular cytogenetics: Genomic imbalances in colorectal cancer and their clinical impact¹

Marian Grade^a, Heinz Becker^a, Torsten Liersch^a, Thomas Ried^b and B. Michael Ghadimi^{a,*}

^a *Department of General Surgery, University Medical Center Göttingen, Germany*

^b *Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, USA*

Abstract. Chromosomal aberrations play a dominant role in colorectal carcinogenesis. The application of fluorescence in situ hybridization (FISH) based techniques such as comparative genomic hybridization (CGH) and spectral karyotyping (SKY) revealed that colorectal carcinomas are characterized by a specific pattern of chromosomal imbalances which sequentially accumulate during cancer progression. This review aims to summarize molecular cytogenetic studies, provides a background on the functional relevance of chromosomal aberrations for colorectal cancer progression and discusses their potential clinical impact.

1. Introduction

Colorectal cancer is the second leading cause of cancer death in Europe and the United States [96, 141]. Despite the stage-dependent application of effective (neo-) adjuvant therapeutic modalities, many patients die due to disease recurrence or metastatic spread [65,73,95,105]. Thus, understanding colorectal cancer progression and establishing reliable biomarkers remains of considerable clinical interest. Damage to the genome is the major mechanism underlying malignant transformation, and chromosomal aberrations are hallmarks of genomic instability and gene deregulation in solid tumors [55,140]. In this review, we will summarize the results of previously published molecular cytogenetic studies, discuss the functional relevance of chromosomal alterations underlying colorectal carcinogenesis and how they impact the clinical course.

¹For this review, we performed a PubMed search (www.pubmed.gov) for the following keywords: “colorectal cancer” and “CGH”, “comparative genomic hybridization”, “SKY”, “spectral karyotyping”, “aCGH”, “array CGH”, “array comparative genomic hybridization”. Publications analyzing ulcerative colitis associated forms of colorectal cancer or case reports were excluded.

*Corresponding author: B. Michael Ghadimi, MD, Department of General Surgery, University Medical Center, Robert-Koch-Str. 40, 37075 Göttingen, Germany. E-mail: mghadim@uni-goettingen.de.

2. Spectral karyotyping (SKY)

2.1. Background

The principle of in situ hybridization was introduced by Gall and Pardue in 1969 [45]. Even though initially performed with radioactively labeled probes, Rudkin and Stollar developed fluorescent detection formats [124]. In 1986, Cremer and colleagues published the concept of interphase cytogenetics [29], which enabled the analysis of non-dividing cells or fixed tissue samples and therefore allowed the simultaneous assessment of chromosomal aberrations, cellular phenotype and tissue morphology [120]. However, FISH is limited to detect specific chromosomal regions or chromosomes. In order to overcome this drawback, spectral karyotyping (SKY) and M-FISH were developed [128, 132]. Whereas conventional cytogenetics is based on a black-and-white banding pattern, SKY visualizes chromosomes of dividing cells based on a multi-color FISH experiment [128].

2.2. SKY analyses

Due to technical difficulties encountered during tissue culture and to the sheer number of cytogenetic abnormalities, SKY has been mainly applied to study cell lines. A graphical synopsis of published SKY analyses

can be found online [104]. In the first published SKY study on colorectal cancer, diploid and/or tetraploid cell lines SW48, HCT116 and DLD-1 as well as aneuploid cell lines SW837, SW480, LoVo, HT-29, COLO-201, T-84 and Caco2 were analyzed [48]. Diploid cell lines only exhibited a few structural aberrations, for instance translocation t(1;6), and duplications dup(2p) and dup(11p). Whole chromosome or chromosome arm aberrations could not be detected. In contrast, aneuploid cell lines revealed frequent numerical aberrations, affecting entire chromosomes as well as whole chromosome arms. These specific cytogenetic abnormalities in the aneuploid cell lines were correlated with centrosome amplification and centrosome instability. A similar analysis of eight human colon cancer cell lines confirmed that DNA amplification and chromosomal translocations are accompanied by chromosomal instability [137]. Melcher and colleagues screened SW480 and SW620 and compared the observed chromosomal aberrations with those reported by conventional G-banding [91]. Using SKY, the authors redefined several chromosomal aberrations, and delineated complex rearrangements that had not been reported, such as der(16)t(3;16;1;16;8;16;1;16;10) and der(18)t(18;15;17)(q12;p11p13;??) in SW620 and der(19)t(19;8;19;5) in SW480. Abdel-Rahman and colleagues investigated 17 colorectal carcinoma-derived cell lines, either replication error (RER) negative (microsatellite stable, MSS, MSI-) or positive (microsatellite unstable, MSI+) [2]. The nine MSI- cell lines revealed gains of chromosome arms 7p, 7q, 8q, 13q and 20q, as well as losses of chromosome arms 17p, 18q and 8p. Furthermore, they observed a variety of mostly unbalanced translocations and a pronounced inter-metaphase variation, with chromosome numbers of 48 to 90. The eight MSI+ cell lines preferably showed a near-diploid pattern, with a few or no rearranged chromosomes. Interestingly, two MSI+ cell lines, LS411 and HCA7, exhibited a high number of altered chromosomes. Furthermore, HCA7 showed six reciprocal translocations. Since epithelial tumors such as colorectal cancers are usually affected by nonreciprocal translocations, the authors proposed an alternative genomic instability pathway. These findings have been partially confirmed by Melcher and colleagues, who analyzed three microsatellite stable (MSS, MSI-) and three microsatellite unstable (MSI+) tumors [92]. They observed two to three aberrant chromosomes in the MSI+ cell lines, and nine to 17 in the MSI- cell lines. Additionally, MSI+ cell lines only rarely displayed complex structural aberrations. Recently,

the same group reported the establishment of a new colonic adenoma cell line, GEKI-2, which is microsatellite stable and reveals no chromosomal aberrations according to SKY analysis [93].

Roschke et al. analyzed colorectal cancer cell lines HCT-116 and HT-29 in order to investigate the consequences of structural and numeric instability on karyotypic progression [122]. Using CGH, FISH and SKY, the authors demonstrated a relative stability of the consensus karyotype over many generations. Interestingly, they did not detect any new clonal structural aberration. Bartos and colleagues analyzed the intratumor heterogeneity in colorectal carcinomas [14]. The authors found that two separate clones from the same tumor exhibited different number of chromosomes and different translocations. Just recently, it has been suggested that (colorectal) adenocarcinoma cell lines and squamous cell carcinoma cell lines are characterized by different mechanisms of centromeric instability [62]. Using SKY, array CGH and FISH, the authors demonstrated that the former exhibited fewer whole arm translocations than the latter. Furthermore, chromosomes of squamous cell carcinoma cell lines with whole arm translocations contained centromeric material from both participating chromosomes. SKY has also been used to analyze carcinogen-induced colonic tumors in mice [54].

3. Comparative genomic hybridization (CGH)

3.1. Background

As mentioned above, preparation of high quality metaphase chromosomes remains a general problem in epithelial tumors. In 1992, Kallioniemi and colleagues introduced a whole genome screening technique termed comparative genomic hybridization (CGH), which allows visualizing chromosomal imbalances based on a two-color FISH experiment with differentially labeled test and reference genomes [71]. As a major advantage, preparation of tumor metaphase chromosomes is not required.

3.2. CGH analyses

3.2.1. Chromosomal imbalances and genomic instability

Based on genomic instability, colorectal carcinomas can be basically categorized into two major types, affecting either chromosome number or structure (chro-

mosomal instability, CIN) or microsatellite sequences (microsatellite instability, MSI) [1,67,81,135]. Accordingly, several investigations have been conducted to elucidate the genomic differences of these subtypes. Schlegel and colleagues published the first CGH study of colorectal carcinoma cell lines and demonstrated that MSI+ tumors generally did not exhibit chromosomal aberrations, whereas CIN+ tumors frequently showed gains of chromosomes 7, 13 and 20q as well as losses of chromosomes 9p, 17 and 18 [127]. Results from other investigators as well as our laboratory confirmed these findings [48,74]. Curtis and colleagues established primary colorectal carcinomas as subcutaneous xenografts by dorsal implantation in severe combined immunodeficient (SCID) mice and could show that the overall number of chromosomal aberrations was much lower in MSI+ cancers than in CIN+ cancers [31].

Other CGH analyses, however, revealed a potential third genetic pathway, exhibiting near-diploid karyotypes accompanied by stable microsatellites (microsatellite and chromosome stable, MACS or CIN−/MSI−) [24,47]. Li and colleagues even hypothesized the existence of tumors revealing both types of instability (CIN+/MSI+) [85]. Analyzing 39 MSI+ carcinomas and 20 CIN+ cancers, the authors observed that 31% of the MSI+ carcinomas showed chromosomal copy number changes, even though none of these aberrations exceeded average values of 25%. Camps and colleagues demonstrated that chromosomal instability and microsatellite instability coexist in highly metastatic derivatives of the colon cancer cell line KM12 [22]. Analyzing 16 MSI− (MSS) and 15 MSI+ primary colorectal tumors, the same group recently confirmed that chromosomal copy number changes do not exclusively occur in MSI− tumors [23].

3.2.2. Chromosomal imbalances and tumor development

Since colorectal carcinogenesis is driven by an accumulation of genetic events [9,82,119,139], several CGH studies evaluated the chromosomal imbalances underlying tumor development and progression. In the first published CGH analysis that focused on the progression aspect, microdissected DNA from normal colorectal mucosa, low-grade and high-grade adenomas and carcinomas was analyzed [118]. The authors demonstrated that the stepwise accumulation of genetic aberrations included gains of chromosomes 1, 7, 8q, 13 and 20 as well as losses of chromosomes 4, 8p and 18q. A similar study by Meijer and colleagues confirmed these findings [89]. In a more de-

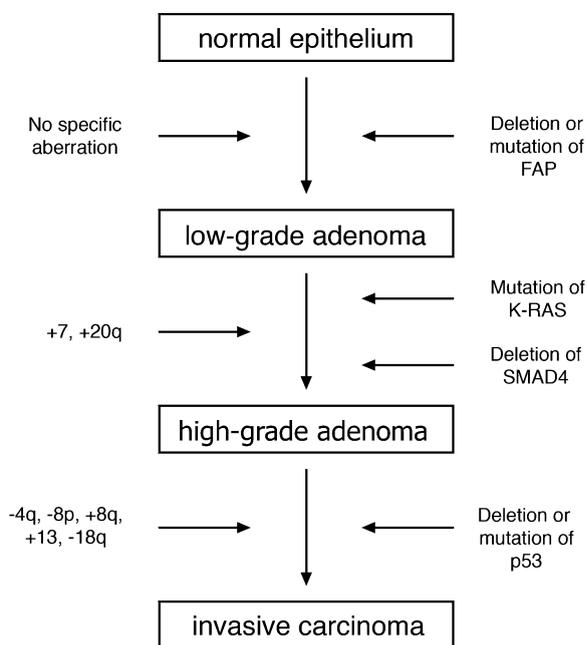


Fig. 1. Chromosomal aberration based progression model of colorectal carcinogenesis. The progression of low-grade colorectal adenomas to high-grade adenomas is accompanied by gains of chromosomes 7 and 20q, whereas gains of chromosomes 8q and 13 as well as losses of chromosomes 4p, 8p and 18q indicate transition into invasive carcinomas (left part). The right part illustrates the genetic changes (gene level) underlying colorectal carcinogenesis as originally proposed by Fearon and Vogelstein [140] and modified according to recent reports [9,82].

tailed analysis of colorectal cancer progression, the authors screened 46 non-progressed adenomas (without invasion), 46 progressed adenomas (presence of a focus of carcinoma) and 36 colorectal cancers and detected seven cancer-associated genetic aberrations: losses of 8p21-ter, 15q11-21, 17p12-13 and 18q12-21, and gains of 8q23-qter, 13q14-31 and 20q13, respectively [61]. Based on these results, we propose a chromosomal aberration based progression model of colorectal carcinogenesis (Fig. 1).

These chromosomal imbalances have been recently confirmed for Thai [112] and Chinese patients [57] as well. Since the genetic changes underlying colorectal cancer progression are primarily based on the analysis of polyploid tumors, Postma and colleagues recently studied flat, nonpolyploid colorectal lesions [114]. They could show that flat adenomas (high-grade dysplastic) as well as flat carcinomas exhibited a similar pattern of chromosomal aberrations as observed in polyploid adenomas and carcinomas. Furthermore, flat colorectal lesions are characterized by a high prevalence of 18q losses and 20q gains. In con-

trast, Richter and colleagues previously detected different chromosomal imbalances [117]. Nonpolyploid adenomas showed higher frequencies of copy number changes. Furthermore, gains of chromosomes 2q, 5, 6, 8q and 12q as well as losses of chromosomes 17p and 20 occurred exclusively in nonpolyploid adenomas. Nonpolyploid carcinomas exhibited frequent losses of chromosomes 8p, 12q, 14, 15q, 16, 17p and 22 as well as gains of chromosomes 3q, 5, 6, 7, 8q, 12q and 13. However, it should be noted that Richter and colleagues mainly analyzed low-grade dysplastic lesions [117], whereas Postma and colleagues studied high-grade dysplastic tumors [114].

3.2.3. Chromosomal imbalances and metastases formation

Since colorectal cancer patients primarily die due to metastatic spread, several investigators focused on stage-specific aberrations, i.e. genetic changes that are associated with clinical Dukes' or UICC stage. But the results remain contradictory, especially with regards to genomic changes underlying metastases formation. However, it is important to note that some groups analyzed DNA from primary tumors, whereas others examined corresponding metastatic lesions.

De Angelis and colleagues analyzed 45 Dukes' stage B, C and D tumors and could not significantly correlate any chromosomal aberration with the clinical stage [33]. In contrast, our group demonstrated that gains of chromosome 8q23-24 were significantly associated with lymph node positivity in colorectal cancers [49]. Whereas this aberration was detected in the vast majority of lymph node positive tumors, it was only rarely present in lymph node negative carcinomas of the same T-category. These findings could become more important since Bardi and colleagues recently demonstrated that the presence of structural changes of chromosome 8 was a stronger prognostic factor in UICC stage III colorectal carcinomas than tumor grade [13]. Of note, all three above mentioned studies described a plethora of common genetic changes, such as gains of chromosomes 7, 8q, 13 and 20 as well as losses of 8p, 17p and 18q. These findings are in concordance with a report by Leslie and colleagues [83]. However, in clear contrast to the results published by our group [49], Dukes' stage C tumors were found to have significantly fewer chromosomal aberrations than Dukes' stage B tumors. Furthermore, the authors demonstrated that gains of 20q, 13q and 8q as well as loss of 18q were significantly correlated with mutation of p53.

Analyzing primary Dukes' stage C and D carcinomas and corresponding metastases, Al-Mulla and colleagues found that chromosomal losses of 17p were associated with lymph node metastases, while gains of chromosome arms 6p and 17q were associated with liver metastases [7]. Another study revealed that UICC stage IV tumors exhibited higher frequencies of chromosomal gains of 6q, 7q, 8q, 13 and 20q than UICC stage II and III tumors [100]. Paredes-Zaglul and colleagues analyzed UICC stage I, II and IV tumors as well as metastatic lesions [109]. The authors stated that liver metastases more frequently showed gains of chromosomes 8q and 13 as well as losses of 4q, 8p, 15q, 17p, 18q, 21q and 22q. Furthermore, losses of chromosomes 9q, 11q and 17q were unique to metastatic lesions. Recent work by our group confirms that tumors with synchronous liver metastases exhibited significantly more chromosomal losses than non-metastatic tumors [Ghadimi et al., unpublished data]. A study by Aragane and colleagues could not detect any significant correlation between UICC classification and chromosomal aberrations, even though gains of 8q23-24 and 20q as well as losses of 18q12-23 were present at higher frequencies in metastatic tumors [8]. However, the potential role of chromosome arm 20q gains for metastatic disease has been confirmed by other investigators. Iwamoto and colleagues observed gains of chromosome arm 20q in 16 out of 18 distant metastases [68]. Korn et al. detected chromosomal gains of 20q in 85% of the analyzed liver metastases, along with gains of 7p, 8q and 13 as well as losses of 1p, 8p, 18p and 18q [79]. Almost identical results have been published by other groups, observing losses of chromosomes 4q, 8p, 17p and 18 as well as gains of 7, 8q, 13 and 20 in liver metastases [35, 111]. These aberrations have also been identified in local recurrences and peritoneal carcinomatoses [36]. Analyses of 10 primary carcinomas, 14 local recurrences, seven peritoneal carcinomatoses and 42 liver metastases revealed that the metastases exhibited the highest frequency of chromosomal aberrations. Furthermore, gains of chromosome arms 5p and 12p were more common in the carcinomatoses. Knosel and colleagues, analyzing 24 primary tumors and 30 metastases of 54 patients, even found gains of chromosome 20q in 100% of cases [75]. Furthermore, chromosomal regions 7q12-11.2, 16p11-12, 19p13, 9q34, 19q13, 13q34, 13q13, 17q21, 22q11, 8q24 and 1q21 were frequently gained, whereas chromosomal losses could be mapped to 18q21-23, 4q27-28, 4p14, 5q21, 1p21-22, 21q21, 6q16, 3p12, 8p24-21, 9p21, 11q22 and 14q13-

21. The same group demonstrated that hematogenous metastases contained more deletions of chromosomes 1p, 3, 4, 5q, 10q, 14 and 21q21 as well as gains of chromosomes 1q, 7p, 12qter, 13, 16 and 22q than lymph node metastases [77]. Furthermore, hepatic metastases more often exhibited deletions of chromosomes 2q, 5q, 8p, 9p, 10q and 21q21 as well as chromosomal gains of 1q, 11, 12qter, 17q12-21, 19 and 22q than their corresponding primary tumors. These differences are in concordance with an analysis of 20 paired samples of primary carcinomas and corresponding synchronous or metachronous metastases [134]. Alcock and colleagues analyzed microdissected sub-regions from primary UICC stage IV tumors and corresponding hepatic metastases [5]. The authors could show that none of the two samples from one case exhibited identical aberrations, although common changes like gains of X and 12q as well as losses of 8p, 16p, 9p, 1q, 18q and 10q were identified.

In order to identify genetic differences between primary colorectal carcinomas and their corresponding lung metastases, Jiang and colleagues analyzed 18 paired samples of primary carcinomas and pulmonary metastases [69]. The authors detected very similar genomic aberrations, with frequent gains of chromosomes 7q, 8q, 13q and 20q as well as losses of 8p, 18p and 18q. However, pulmonary metastases contained a higher total number of chromosomal copy number changes, especially losses of 4q and 8p, respectively. Knosel and colleagues could partially confirm these findings, with particularly more chromosomal deletions at 3p, 8p, 12q, 17q and 21q21 as well as chromosomal gains at 5p in the lung metastases [78]. Furthermore, the authors identified higher frequencies of chromosomal losses of 1p, 3p, 9q, 12q, 17q, 19p and 22q as well as gains of 2q, 5p and 6 in pulmonary metastases compared to liver metastases.

3.2.4. Chromosomal imbalances and patient survival

Other investigators aimed to explore a potential influence of specific genomic aberrations on patient survival. Rooney and colleagues screened 29 Dukes' stage C cancers [121]. Interestingly, they could not detect any genetic alteration that was present in more than one third of all cases. Additionally, none of the observed aberrations was associated with patient's survival. In contrast, De Angelis and colleagues demonstrated that losses of chromosomes 1p, 4q, 8p, 14q or 18q as well as gains of 20q resulted in shorter survival times [34]. Knosel and colleagues, however, analyzed tumors from 37 patients and could show that gains of 2p14-15, 6q23-24, 15q22-23, 22q11.2 and losses of

1p36.1-36.2, 4q31.3, 4q35, 8q12-21, 8p11.2 and 9p22 were associated with shorter disease-specific survival, whereas gains of 20q13.3 and losses of 18q11.2 were associated with longer disease-specific survival [76].

4. Array-based comparative genomic hybridization (aCGH)

In the past few years, CGH has been advanced through replacing the metaphase chromosomes (chromosome or metaphase CGH) with probes spotted on arrays (array-based comparative genomic hybridization, aCGH) [110,131]. This resulted in a higher resolution if compared to conventional CGH. BAC arrays, oligonucleotide arrays and cDNA arrays constitute platforms commonly used for hybridization. To date, just a few studies have been published analyzing colorectal cancers. Nakao and colleagues surveyed 125 colorectal cancers with an array consisting of 2463 clones [101]. High-frequency losses ($\geq 35\%$) affected chromosomes 5q, 8p, 17p, 18p, 18q and 20p, whereas high-frequency gains mapped to chromosomes 7p, 7q, 8q, 11q and 20q, which is basically consistent with the published data on metaphase CGH, as mentioned above. However, the authors identified additional aberrations that have not been previously reported. High-level amplifications could be mapped to four distinct loci on chromosome 8 and six loci on chromosome arm 20q. However, 95% of the altered clones were gained or lost at low frequencies ($< 35\%$). Interestingly, DNA copy number alterations in seven microsatellite unstable tumors (MSI+) mainly affected chromosome 8q. Of note, the authors could not detect chromosomal aberrations that significantly differed between tumors of different clinical phenotypes. Douglas and colleagues investigated copy number changes in 48 colorectal cancer cell lines and 37 primary colorectal cancers, consisting of seven MSI+ and 30 CIN+ tumors [40]. Even though cell lines and primary tumors exhibited very similar aberrations, such as gains of chromosomes 7, 8q, 13 and 20 as well as losses of chromosomes 8p and 18q, losses of chromosome 6 and gains of chromosomes 12p and 15 were more common in cell lines. Comparison of CIN+ and MSI+ cancers revealed that the former displayed significantly more aberrations than the latter. In particular, gains of chromosome 20 as well as losses of chromosomes 8p, 17p and 18q appeared more frequently in CIN+ cell lines/cancers. Losses of chromosome 18q21.1-21.2, however, were predominantly found in MSI+ cell

lines/cancers. Interestingly, 8q amplifications mapped to 8q24.21 in CIN+ samples, but to 8q24.3 in MSI+ samples. The observation that microsatellite-unstable (MSI+) tumors carry fewer chromosomal aberrations than microsatellite-stable (MSI-, MSS) tumors has been recently confirmed for colon cancers [142]. Jones and colleagues screened 23 microsatellite stable, near diploid tumors (MSI-/CIN-) in order to explore if they represent a discrete group besides microsatellite unstable (MSI+) and chromosomal unstable (CIN+) cancers [70]. The authors discovered that these tumors comprised a heterogeneous group. Some tumors exhibited very few chromosomal aberrations (≤ 6), whereas some showed more than 10, mainly affecting chromosome arms 1p, 5q, 8p, 13q, 17p, 18q and chromosome 20. Furthermore, comparison with previously published results on CIN+ and MSI+ tumors demonstrated that the MSI-/CIN- tumors revealed low frequency gains of chromosome arms 9p and 19p, infrequent losses of 5q and high frequency gains of 20q, respectively. Davison and colleagues analyzed 48 colorectal cancer cell lines and 37 primary colorectal cancers using a genomic microarray covering a 4.61 Mb region of chromosome band 20p12.1 [32]. They observed that 55% of the cell lines and 23% of the primary tumors showed a recurrent ~190 kb deletion, which might lead to relevant tumor suppressor genes.

Only recently, it has been demonstrated that array-based CGH might be of value for prediction of survival after hepatic metastases resection [88]. The authors analyzed 50 liver metastases using an array consisting of 2463 bacterial artificial clones and correlated follow-up data with the total fraction of genome altered (FGA). Interestingly, patients with a high (>20%) FGA had a median survival time of 38 months compared with 18 months in patients with a low FGA.

5. Relevance of chromosomal aberrations in solid tumors

Do the observed chromosomal imbalances just appear randomly by chance during tumor formation? This is still a matter of much debate [4,17,43,44,84,86,90,115,116,126,133]. From our point of view, evidence is accumulating that numerical chromosomal aberrations are a driving force during carcinogenesis. *Firstly*, it has been demonstrated that colorectal carcinomas are defined by a specific distribution of recurrent chromosomal imbalances [37,61,89,118,119]. For instance, there is no other solid tumor in which a

gain of chromosome 13 is as prominent as in colorectal cancers [104]. *Secondly*, specific chromosomal aberrations are maintained in advanced lesions like metastases or recurrent tumors, although colorectal cancers are characterized by a substantial clone to clone variability, i.e. genetic instability. And even long-term *in vitro* tissue culture of cancer cells does not alter the tumor specific distribution of genomic imbalances, indicating a remarkable stable genotype, at least with respect to these chromosomal gains and losses [48,97,104,144]. *Thirdly*, distinct copy number changes of specific chromosomes have been identified as predictors of lymph node positivity [49] and clinical outcome [13,143]. *Fourthly*, several investigations provide strong evidence that genomic imbalances directly impact the cellular transcriptome of cancer cells, therefore supporting a role for aneuploidy in tumorigenesis in addition to the transcriptional and mutational deregulation of oncogenes and tumor suppressor genes. We could previously demonstrate that the experimental insertion of one chromosome via microcell-mediated chromosome transfer resulted in a significant increase in the average transcriptional upregulation of genes on the trisomic chromosomes [138]. Only recently, we explored the relationship between chromosomal aneuploidy and average gene expression levels in patients with rectal adenocarcinomas using oligonucleotide arrays and comparative genomic hybridization [53]. For those chromosomes frequently gained or lost in rectal cancers, we were able to identify a strong positive correlation between the chromosome arm copy number and the average transcriptional activity of its resident genes. Results for the six arms with very strong correlations and a significance of $p < 0.05$ are illustrated in Fig. 2. Tsafirir and colleagues could also demonstrate that frequently altered chromosomal regions in colon cancers are gained and overexpressed (7p, 8q, 13q, 20q), whereas others are lost and underexpressed (1p, 4, 5q, 14, 15q, 18) [136]. A similar picture emerged from other reports of primary tumors and cancer cell lines [3,66,98,113].

6. Summary

Colorectal carcinoma is probably the most intensively studied solid human tumor, and molecular cytogenetics played a pivotal role in evaluating the underlying chromosomal imbalances. SKY analyses confirmed the karyotypic complexity of colorectal cancers and revealed that DNA amplification and chromosomal

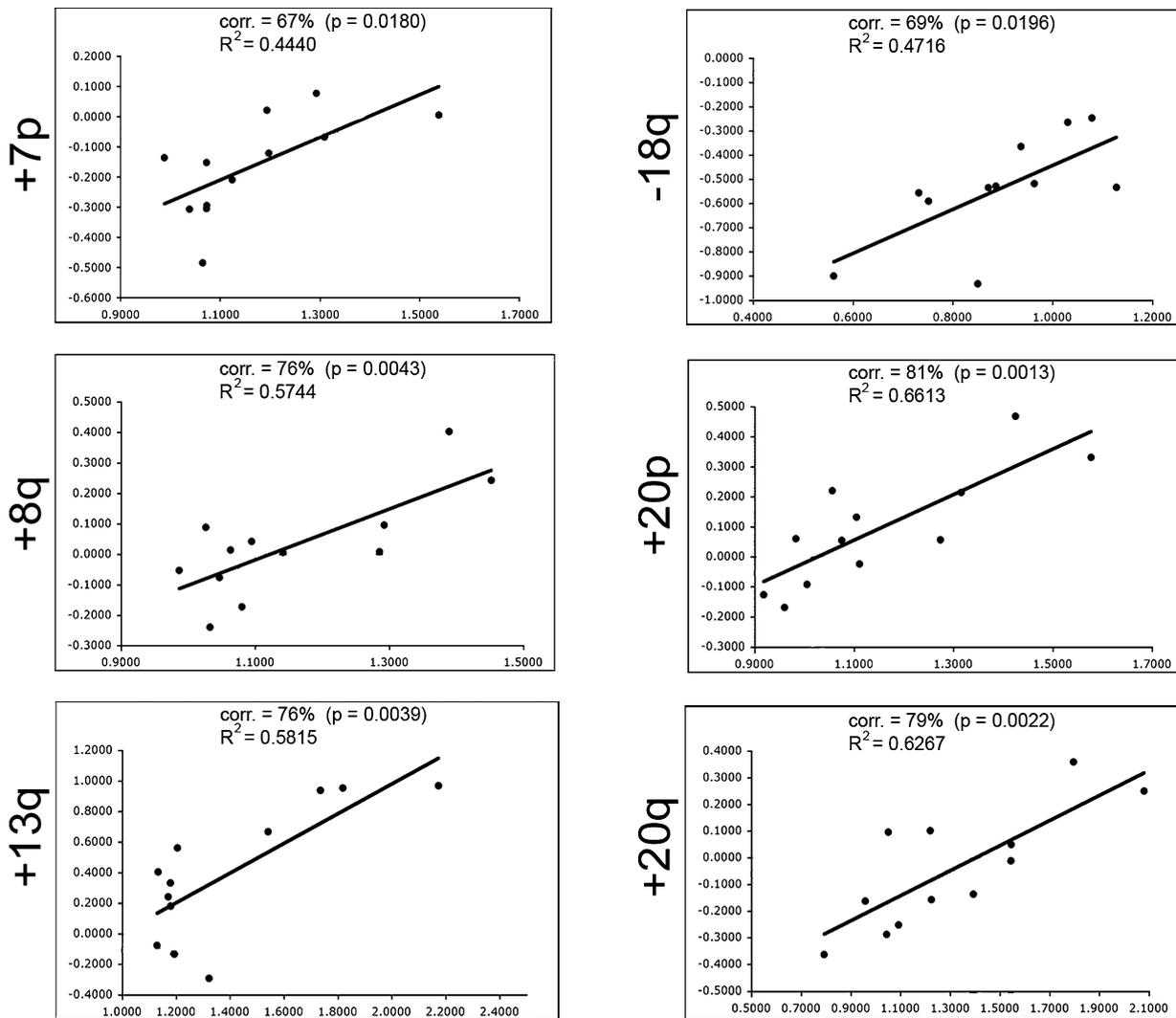


Fig. 2. Correlation of chromosomal copy numbers and resident gene expression levels for frequently aneuploid chromosome arms. As previously described [53], the average chromosome arm expression levels (relative to a reference RNA pool) were computed for 12 rectal adenocarcinomas. Similarly, the CGH values for these tumors were measured along the length of each chromosome arm and averaged in order to compute an average copy number for each chromosome arm. In this figure, the average gene expression value (y -axis) is plotted against the average CGH ratio value (x -axis) for each patient. An x -value of 1 corresponds to a normal (diploid) average copy number, whereas a y -value of 0 indicates an equal average expression between tumor and reference pool. The percentage correlation, its p -value and the R^2 are shown in each plot. The directionality of the typical copy number change in colorectal carcinomas is represented as a + (gain) or - (loss) preceding the chromosome number. The strong positive correlation at significance of $p < 0.05$ for these chromosomal arms is clearly depicted.

translocations are accompanied by chromosomal instability. Furthermore, SKY redefined several previously misclassified chromosomal aberrations and delineated highly complex rearrangements and balanced translocations. The application of CGH established solid evidence that colorectal tumorigenesis requires the acquisition and maintenance of a specific distribution of genomic imbalances, such as gains of chromosomes 7, 8q, 13 and 20q, as well as losses of 4q, 8p, 17p and 18q, respectively (Fig. 3). Several FISH studies confirmed

the involvement of specific loci [11,18,19,27,38,39,46, 51,58–60,72,102,103,107,108], for example the relevance of chromosomes 8q11–24 [20] and 20q13 for metastases formation and prognosis [11,63,79]. However, for advanced stages of this disease, i.e. metastases formation, the results of previously published studies remain contradictory.

Only recently, introduction of array-based comparative genomic hybridization dramatically improved the resolution of conventional chromosome CGH. This

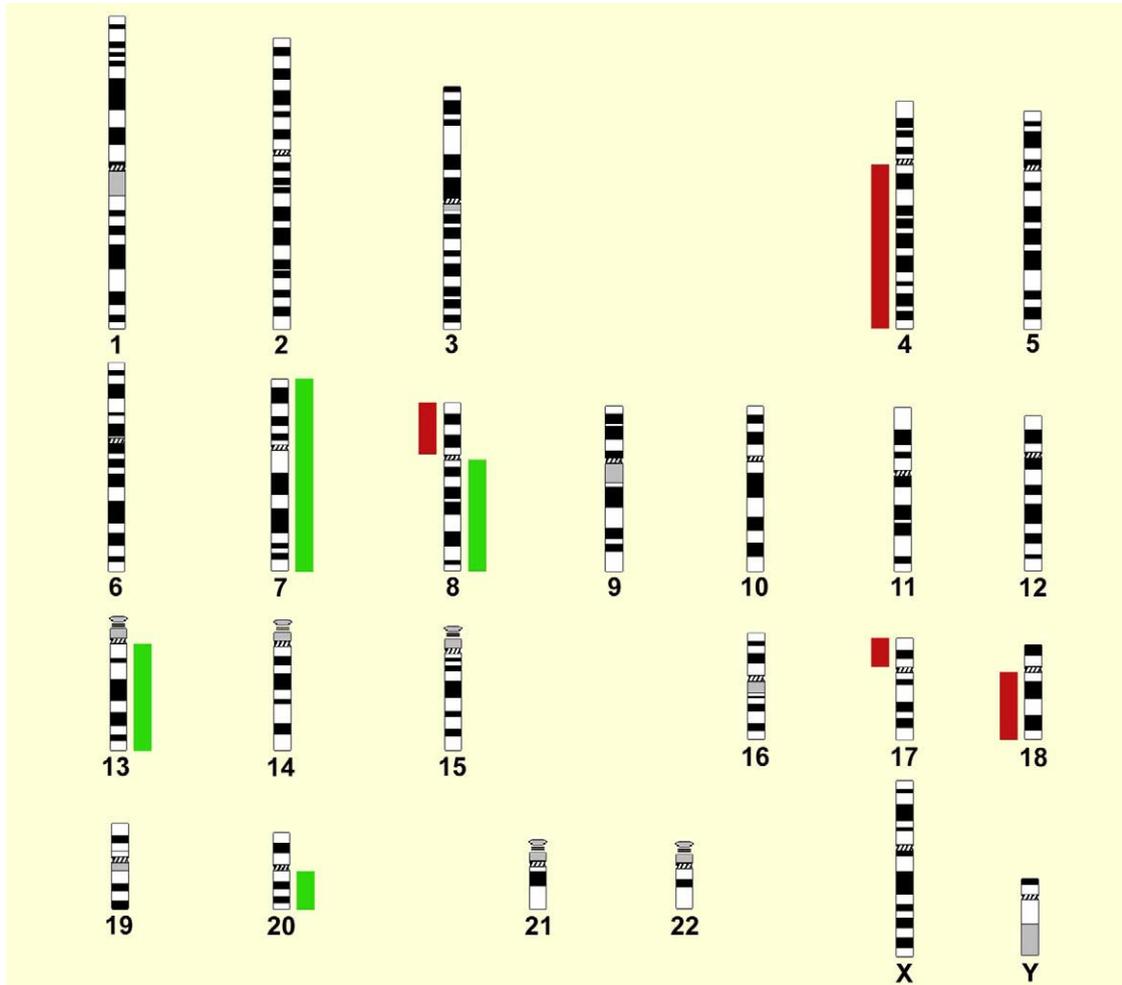


Fig. 3. Specific and recurrent pattern of chromosomal gains and losses in colorectal carcinomas. These aberrations represent the most frequently observed chromosomal imbalances detected with metaphase CGH (for details, see Section 3). Vertical lines on the left of each chromosome ideogram represent genetic losses in the tumor, whereas those on the right correspond to a chromosomal gain.

technique also allows a direct mapping of chromosomal aberrations since the genomic sequence of the spotted probe is known. Furthermore, if oligonucleotide arrays are used, DNA copy number changes can be directly compared to gene expression levels, because this platform has originally been designed for expression profiling.

7. Future perspective

From the clinical point of view, the translation of basic research into clinical practice (from bench to bedside) remains problematic for colorectal cancers. Even though molecular cytogenetics established the underlying chromosomal imbalances of colorectal car-

cinogenesis, reliable molecular markers for diagnosis, prognostication and therapy stratification are still lacking [6,52]. In clear contrast, the successful clinical application of Imatinib (Gleevec[®]) in the treatment of chronic myeloid leukemia is primarily based on the cytogenetic discovery of an aberrant translocation between chromosomes 9 and 22, i.e. the Philadelphia chromosome [106,123]. The observation that this translocation results in an aberrant fusion product, the BCR-ABL tyrosine kinase [16], led to development and establishment of Imatinib, which targets this tyrosine kinase [41,42]. Furthermore, FISH became a routine diagnostic tool in breast cancer, since the application of the monoclonal antibody Trastuzumab (Herceptin[®]) requires identification of tumors as HER2/neu positive [21]. FISH with fluores-

cently labeled HER2/neu probes allows convenient determination of receptor status [56]. Additionally, based on the results of Sokolova and colleagues, a multitarget multicolor FISH assay (UroVysionTM) can be applied to detect aneuploidy for chromosomes 3, 7 and 17 as well as losses of 9p21 in urine specimens from patients with transitional cell cancers of the bladder [130].

Therefore, the question remains whether there is a clinical value of molecular cytogenetics for colorectal carcinomas. We hypothesize that, as soon as chromosomal aneuploidies will be tightly correlated to disease prognostication, and as soon as rational therapies targeting a specific genetic pathway (or pathways) will be developed, interphase FISH with selected DNA probes will become indispensable for individualized disease management. One can speculate that FISH might be of value for determining the epidermal growth factor receptor (EGFR) status prior to therapy planning. In 2004, Cunningham and colleagues demonstrated that cetuximab (Erbix[®]) in combination with irinotecan has significant activity in patients with irinotecan-refractory colorectal cancer [30]. However, the authors did not observe an association between EGFR expression, i.e. staining by immunohistochemistry (IHC), and response to therapy. This finding is in concordance with data published by Saltz and colleagues, also reporting a poor correlation between EGFR score and response to cetuximab monotherapy in patients with refractory colorectal cancer [125]. Chung and colleagues recently reported that four of 16 EGFR negative colorectal cancers showed objective response to irinotecan plus cetuximab [26]. Accordingly, there is much debate on how to best measure the EGFR status [15, 28,94,129]. Of note, it has been demonstrated that the level of EGFR expression varies depending on factors such as fixation or time of storage [10], indicating that it remains problematic to objectively draw definitive conclusions from IHC results. Since DNA (i.e. the EGFR gene) is much more stable than proteins (EGFR protein) FISH might help to overcome these drawbacks. Furthermore, Moroni and colleagues recently reported that cetuximab inhibited proliferation of colorectal cancers with amplified EGFR copy number, but not of tumors with unamplified EGFR copy number [99]. Additionally, increased EGFR gene copy detected by FISH is associated with improved survival after gefitinib therapy in patients with bronchioloalveolar carcinoma [64]. To further complicate this debate, two affinity forms of the EGF-Receptor exist [80,87]. Since the commonly available IHC antibodies that target EGFR are not receptor-specific, interphase FISH

seems to be an interesting format for enumeration of EGFR copy number status.

However, other clinical questions might require more complex and comprehensive interrogation methods and can probably not be answered by detecting the amplification status of one gene. Such questions include tumor response to therapy or the establishment of individual risk profiles for tumor development. For instance, studies using gene expression arrays recently demonstrated response prediction to preoperative chemotherapy in breast cancers [12,25] and to preoperative chemoradiotherapy in rectal adenocarcinomas [50]. We hypothesize that array-based technologies will play an important role in establishing a "personalized medicine", where patients are treated based on specific biological features of individual tumors.

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